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Research article

Effects of High Intensity Interval Training in Normobaric Hypoxia on Aerobic Performance and Exercise-Induced Motor Performance Fatigue in Young Biathletes

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Abstract

This study investigated the effect of high-intensity interval training (HIIT) in normobaric hypoxia on aerobic performance in young biathlon athletes. In addition, the study aimed to assess the impact of training in hypoxia on the mechanisms of exercise-induced motor performance fatigue. In a randomized, controlled crossover study twelve athletes (age 15.7 ± 1.0 years) completed a HIIT in normobaric hypoxia (hypoxia training) (fraction of inspired oxygen, F_iO_2 = 15.2%) and normoxia (normoxia training) in a randomized order. The HIIT was performed 3 days/week for 6 weeks (3 weeks in hypoxia and 3 weeks in normoxia, with a 3 week wash-out period in between) and consisted of 5 x 4 minutes running (80% of peak oxygen uptake), separated by 3 minutes of active recovery and 4 x 1minute arm cranking (60% peak power), interspersed with a 2 minute rest. Peak oxygen uptake ($\dot{V}O_{2peak}$), hypoxia-inducible factor 1 alpha (HIF1α), vascular endothelial growth factor (VEGF), pro-inflammatory cytokines, muscle damage biomarkers and total antioxidant status were analyzed before and after both training protocols (HT and NT). A significant effect of hypoxia on $\dot{V}O_{2peak}$ (ηp^2 = 0.321, p = 0.044) and hypoxia and training on $\dot{V}O_2$LT and haemoglobin concentrations (ηp^2 = 0.689 p = 0.001) were observed. The $\dot{V}O_{2peak}$ was significantly higher post-HT compared to pre-HT ($p < 0.01$). A significant effect of oxygen conditions and training on the serum post-exercise VEGF (ηp^2 = 0.352, p = 0.033) and myoglobin concentrations (ηp^2 = 0.647 p = 0.001) was found. A significant effect of hypoxia was also observed for cytokines levels: interleukin-6 (ηp^2 = 0.324 p = 0.042), tumour necrosis factor alpha (ηp^2 = 0.474 p = 0.009) and transforming growth factor beta (ηp^2= 0.410, p = 0.018) with a non-significant effect on antioxidant status. This study shows significant differences in the aerobic performance and biomarkers of muscle damage after exposure to hypoxia training. These findings highlight that HIIT in hypoxia is sufficient to enhance aerobic performance and may also reduce skeletal muscle susceptibility to fatigue in young biathletes.

Key words: Hypoxia, biomarkers of skeletal muscle damage, training, biathlon, oxygen uptake, lactate threshold.

Introduction

Biathlon is an endurance sport that combines cross-country skiing with rifle shooting. Depending on the type of competition, a biathlete covers a distance of 6.0 - 15.0 km (females) and 7.5 - 20.0 km (males) and completes two or four shooting bouts (International Biathlon Union - IBU Datacenter, 2019). In biathletes, skiing velocity as well as shooting speed and accuracy are the fundamental factors for competitive performance. However, high aerobic capacity and high physical and mental strength are increasingly important for the overall performance (Armstrong and Welsman, 2019; Jonsson Kårström et al., 2019; Björklund and Laaksonen, 2022). High-intensity endurance training induces numerous physiological adaptations e.g. increased respiratory performance, tissue oxygenation and improved cardiovascular haemodynamics (Jonsson Kårström et al., 2019; Laaksonen et al., 2020). These physiological adaptations increase exercise performance and can accelerate the reduction of post exercise acidosis and inflammation, thus helping the body to quickly return to a pre-exercise state (Sandbakk et al., 2013; Björklund et al., 2022; Staunton et al., 2022).

Accumulating evidence shows that high intensity interval training (HIIT) induces numerous physiological adaptations that improve exercise capacity and metabolic health in athletes (Abe et al. 2015, Buckley et al. 2015, Park et al. 2022). The effect of HIIT depends on the intensity and duration of the exercise training (Coatses et al. 2023). A long duration intervals (5- to 10-minute bouts, total duration of 40 - 45 minutes) of HIIT improve endurance performance and oxygen uptake more than shorter intervals (2- to 4-minute bouts, total duration of 15-20 minutes) at a higher intensity in junior athletes (Sandbakk et al., 2013). It is worth mentioning that the improvements in peak oxygen uptake ($\dot{V}O_2$peak) observed in junior athletes may be less pronounced than those reported in adults exposed to a similar training stimulus (Dotan, 2017).

The effects of HIIT on exercise performance in adolescents engaged in specific biathlon training have not yet been fully elucidated. Previous studies of elite biathletes have shown that skiing performance is partially influenced by higher gross efficacy, $\dot{V}O_2$peak and oxygen uptake at the lactate threshold velocity ($\dot{V}O_2$LT) (Jonsson Kårström et al., 2019). It has been suggested that $\dot{V}O_2$ at 4 mmol/L but not anaerobic capacity estimated by anaerobic metabolic rate and accumulated oxygen deficit, is a more critical determinant of skiing performance (Laaksonen et al., 2020). It is also possible that with long-term training, additional physiological mechanisms may become increasingly important in determining biathletes' skiing performance.

It has been proved that exercise training of elite athletes may induce muscle fatigue characterized by a decreased ability to generate appropriate amounts of muscle

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force during contractile activity, an increased perception of fatigue (Behrens et al. 2023, Finsterer, 2012). Moreover, biathletes skiing performance can be compromised by muscle damage resulting from exercise strain. Following muscle injury, resident cells secrete proinfmmatory molecules such as tumour necrosis factor alpha (TNF-α). The inflammatory response to damage also leads to overproduction of reactive oxygen spices (ROSs) from circulating and residential immune cells. In healthy muscle, ROSs activated signalling pathways essential for proper muscle regeneration. It has been shown that the hypoxia exposition has the potential to enhance muscle regeneration by positively modulating the local and systemic inflammation response.

During high intensity exercise biomarkers of muscle damage might be used to determine mechanisms of exhaustion during exercise in order to detect defective metabolic pathway and fatigue (Gangwar et al. 2020, Faiss et al. 2013). Interleukin-6 (IL-6), a cytokine with both pro- and anti-inflammatory properties (Tilg et al. 1997), is released from the muscle fibres, creating an anti-inflammatory environment by stimulating the secretion of other anti-inflammatory cytokines into the blood and inhibiting the production of the proinflammatory cytokine - tumour necrosis factor alpha (TNF-α). Exercise training is expected to suppress TNF-α long-term, protecting against diseases associated with low-grade chronic inflammation (Petersen and Pedersen 2005). Transforming growth factor beta-1 (TGF-β1), which plasma levels increase transiently during strenuous acute exercise and exercise training (Czarkowska-Paczek et al. 2006, Hering et al. 2002), plays a key role in increasing the production of ROS, which can lead to cell and tissue injury but at the same time stimulate antioxidant defence (Liu and Desai 2015). The release of myokines and other cytokines during exercise appears to have an important role in mediating local and systemic inflammation (Tsukiyama et al., 2017).

The increasing popularity of biathlon has also enhanced the interest in the methods of training optimization, especially in high altitude training (Morrison et al. 2018, Fitzpatrick and Panagodage Perera, 2020). Given that high altitude exposure fosters beneficial physiological responses, we hypothesized that interval training in normobaric hypoxia may also induce beneficial adaptive responses in young biathletes.

Intermittent hypoxia has been used to improve health and athletic performance, nevertheless, the risks associated with hypoxia exposure exist (Pialoux et al. 2009). The intensity, duration, number and frequency of the hypoxic exposures are key determinants of whether the effects on health and performance are beneficial or detrimental (Burtscher et al. 2024; Millet and Girard, 2017; Zhang et al. 2023). It has been previously demonstrated that the participation in HIIT under intermittent normobaric hypoxia improves maximal oxygen uptake ($\dot{V}O_2max$) to a greater extent than the same training regimen in normoxia (Żebrowska et al. 2019). The primary mechanism through which hypoxia training enhances exercise performance is the improvement of the haematological profile and oxygen-carrying capacity via increased erythropoiesis (Tobin et al. 2022, Millet and Girard, 2017). However, several studies have not confirmed an increase in erythropoiesis, which may be due to insufficient duration or intensity of hypoxic exposure. Discrepancies in research findings could be attributed not only to variations in hypoxia protocols but also to individual differences in acclimatization to hypoxia (Baranauskas et al. 2022, Vogt et al. 2001).

Hypoxia modulates the secretion of hypoxia-inducible factor alpha (HIF-1α), which has been identified as the main regulator of expression of the genes involved in the adaptation to hypoxia (Semenza and Wang 1992). For example, HIF-1α mediates the expression of genes encoding glucose transporters, including solute carrier family 2 member 1 (*SLC2A1*), coding for glucose transporter 1 (GLUT1)) and *SLC2A3* (coding for GLUT3), which results in increased glucose uptake and glycolysis (Chen and Gaber, 2021). HIF-1α also induces the transcription of genes encoding vasoconstrictors (e.g. endothelin-1) (Yamashita et al., 2001), proangiogenic factors (vascular endothelial growth factor: VEGF) (Liu et al., 1995), as well as the genes involved in the regulation of anti-inflammatory responses and tissue protection, including TGF-β1 (Turner et al., 2017; Sumi et al., 2018). Adaptive responses to hypoxia, such as those induced by high altitude exposure, have been shown to improve hypoxia tolerance, protect against future hypoxic or ischemic insults (Chacaroun et al., 2017; Burtscher et al., 2022), and enhance exercise performance (Brocherie et al., 2017; Czuba et al., 2019). Also, hypoxic preconditioning attenuates the ischemic-reperfusion injury in young healthy athletes (Jarrard et al., 2021). Although the physiological mechanisms responsible for the beneficial adaptations to hypoxic training have been examined across different sports disciplines in recent decades (Wilber, 2007; Sinex and Chapman, 2015; Camacho-Cardenosa et al., 2021), research on biathletes remains limited (Czuba et al., 2019; Staunton et al., 2022).

We designed this study to investigate the effect of HIIT in normobaric hypoxia on aerobic performance, haematological parameters and hypoxia-induced factors (i.e. HIF-1 alpha, VEGF) and biomarkers of muscle fatigue in young biathlon athletes. We hypothesized that HIIT in hypoxia would induce greater improvements in aerobic performance and enhance protective mechanisms against exercise-induced motor performance fatigue, compared to the normoxic training in young biathletes.

Methods

Study population and sampling procedure

This study was conducted among members of the National Team of the Polish Biathlon Association (Junior Women's Team and Junior Men's Team). It included the entire population of junior athletes from the Regulatory Team of the Polish Biathlon Association during the 2018-2019 season. Sixteen biathletes, pupils of sports championship schools were assessed for eligibility. Four athletes were excluded due to illness (n = 3) and injury (n = 1).

Twelve biathletes (five males and seven females) aged 15.7 ± 1.0 years, with a minimum of 3 years of training experience (mean training status of 4.4 ± 3.1 years) were asked to arrive at the laboratory in the morning hours

in a non-fasted state to have their body mass and body composition measured by means of bioelectrical impedance method (InBody220 Biospace Inc., Seoul, Korea). The experiment was approved by the Ethics Committee of the Academy of Physical Education in Katowice (Ethics Committee decision no. 9/2016) and conformed to the standards set by the Declaration of Helsinki. Written informed consent was obtained from the biathletes' parents/guardians.

As part of their periodic sports examination, all biathletes regularly participated in exercise testing conducted at the Institute of Sport Sciences Laboratory. Consequently, they all had valid medical clearance and demonstrated no contraindications to participating in the study. Both, the athletes and their legal guardians were informed of the study's purpose, procedures, potential risks and benefits. Prior to participation, each athlete completed a health declaration and provided written informed consent. The participants were also informed of their right to withdraw from the study at any time without giving reasons. The inclusion criteria were as follows: (1) good general health, (2) absence of haemodynamic dysfunction or inflammatory disease within the three months preceding the study, (3) membership in the National Team of the Polish Biathlon Association, (4) a minimum of three years of training experience, (5) non-smoking status and (6) no use of medications or supplements that could influence the study outcomes.

The study participants were instructed to abstain from exercise, caffeine, alcohol, vitamins, and any medicines for 24 h before exercising and were placed on a standardized normocaloric diet for the duration of the study. The recommended daily intakes of macronutrients were as follows: proteins: 12-15%, carbohydrates: 55-65%, and fats: 25-30% (US Department of Health Human Services 2019). All participants were born and resided at sea level.

Study design

Two days before the start of the exercise testing, participants were familiarized with the study procedures. Each participant performed the following protocol: pre-training graded exercise tests – running (REx1 and REx3) and arm cranking exercise (AEx) in normoxia and normobaric hypoxia, a 3-week HIIT programme in normoxia (normoxic training: NT) and a separate 3-week HIIT in normobaric hypoxia (hypoxic training: HT), with a 3-week wash-out period in between, and post-training graded running exercise tests (REx2 and REx4) in normoxia after the completion of each respective training programme (NT ad HT) (Figure 1).

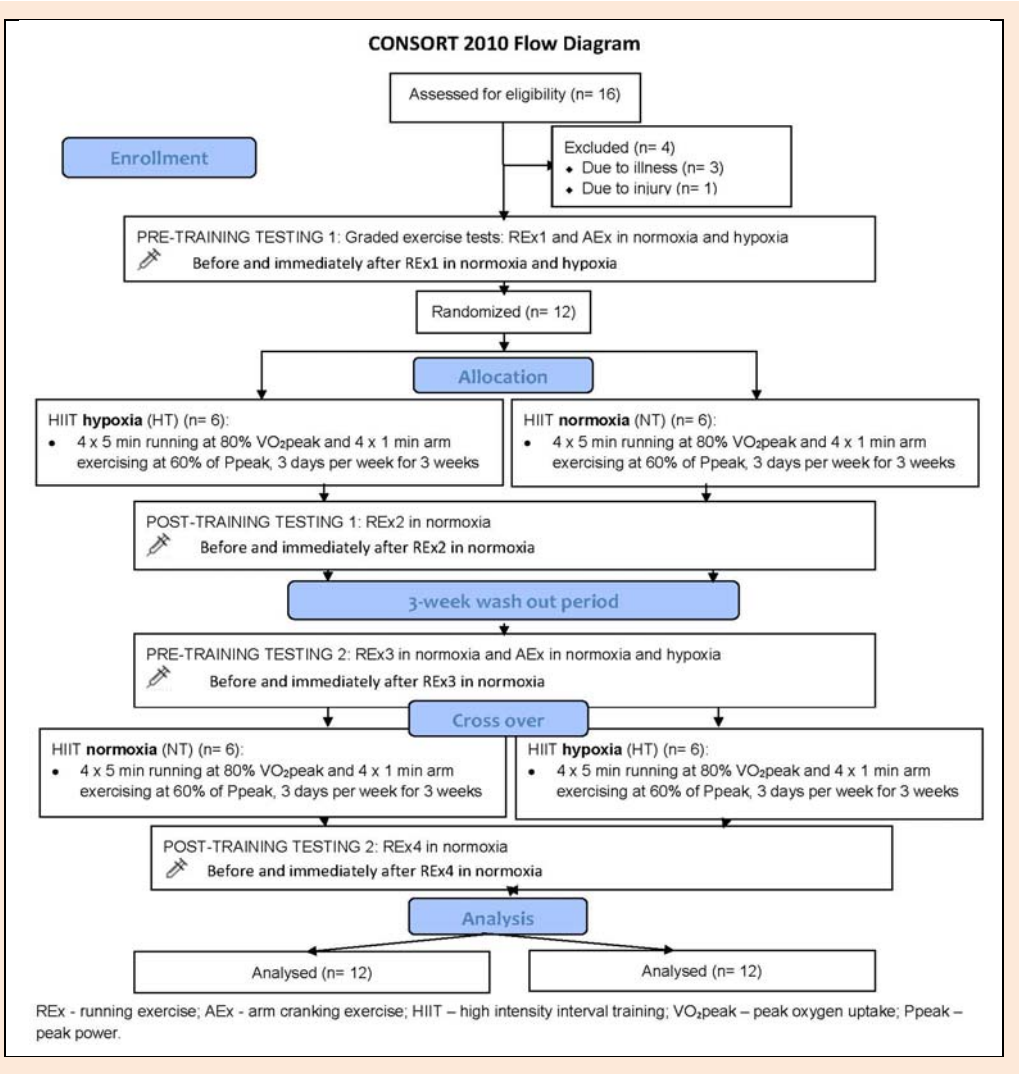

Figure 1. Study protocol.

Normobaric hypoxia was defined by FIO_2 = 15.2%, which was corresponding to 2,600 m altitude and p = 990 hPa. The hypoxic environment was simulated using a HYP-123 Hypoxic Generator (LOWOXYGEN Technology GmbH, Berlin, Germany). Normoxia was characterised by FIO_2 = 20.9% and p = 990 hPa. The study protocol was based on previously conducted laboratory studies under controlled ambient conditions (21°C, 60% relative humidity) (Żebrowska et al., 2019; Czuba et al., 2019).

Pre-training graded exercise tests in normoxia and normobaric hypoxia (Pre-training testing)

At baseline, all subjects performed a standard graded running exercise test (REx1, REx3) to measure their individual aerobic performance ($\dot{V}O_2$peak), lactate threshold (LT), $\dot{V}O_2$/LT, and biochemical variables, including blood lactate concentrations (BLA), biomarkers of muscle damage and oxidative stress. For determination of the BLA, blood samples were taken in the last 30 seconds of each work rate. The Dmax method (Cheng et al. 1992) was used to determine the individual lactate threshold (LT). Additionally, all participants performed an arm cranking exercise test (AEx) to measure their individual peak power (Ppeak). REx1, REx3 and AEx were performed in normoxia and normobaric hypoxia. $\dot{V}O_2$peak and Ppeak were used to determine the intensity of HIIT protocols. The order of the graded tests was randomly assigned to participants, with a minimum of three days between the sessions to prevent any potential interference with exercise capacity (Fig. 1). The test under hypoxia and normoxia was used to determine the training load in the same condition.

Graded REx started at a speed of 6 km/h and 0% inclination; the speed was then increased every 3 min by 2 km/h until 12 km/h. The inclination was elevated every 3 min by 2.5% until a maximal individual load was reached. Oxygen uptake ($\dot{V}O_2$), pulmonary minute ventilation ($\dot{V}E$), and heart rate (HR) were continuously monitored from 6 min before the onset of the test and during the exercise using a respiratory analyzer (Matalyzer 3B, Cortex, Germany). The maximal performance was considered when three of the following criteria were fulfilled: respiratory exchange ratio ≥ 1.1 (RER ≥1.1), $\dot{V}O_2$ plateau at peak exercise, HR peak ≥ 90% of the estimated maximal HR (220-age), and BLA ≥8.0 mmol/l (Cunha et al., 2010). Graded AEx was performed in a standing position using an arm ergometer. The test started with a 3-min warm-up at an intensity of 30 W. The intensity was then increased every min by 30 W up to the individual maximal exercise intensity (Ppeak) and HR peak ≥ 90%. HR was continuously monitored (FT2, T31-Coded, Polar, Kempele, Finland) during each exercise test.

Training programme

In a randomized, controlled crossover study, biathletes completed a 3-week HIIT programme under normobaric hypoxia and normoxia in a randomized order. Participants performed 80-min HIIT sessions three times per week. Each HIIT session consisted of a warm-up, HIIT exercise, and a cool-down. A 10-min acclimatization in a seated position preceded the warm-up, which consisted of a 10 min walking at the velocity of 6 km/h. The HIIT exercise session comprised four 5-minute bouts of running at an intensity of 80% $\dot{V}O_2$peak, interspersed with 3 min of walking after each bout of running, and four 1-minute bouts of arm exercise at an intensity of 60% Ppeak, performed on an arm ski ergometer, interspersed with a 2-min rest and performed after a 10-min cool down following the high intensity running. The exercises were designed to replicate the movement pattern specific for biathlon.

After completion of one of the interval training protocols (NT or HT), participants were asked to arrive at the laboratory the following day for a graded exercise test (please refer to section 2.2.3) and then rested for 3 weeks before attending the next interval training (NT or HT).

The training sessions took place in an environmentally controlled chamber and during the sessions the atmospheric conditions i.e. temperature, humidity and concentration of oxygen were controlled.

Pulse oximetry was used to measure peripheral blood oxygenation (SpO2) (Pulse Oximeter, Braun, Xuzhou Yongkang Electronic Science Technology Co., Ltd., China) and arterial blood pressure (SBP/DBP) were measured in duplicate with a sphygmomanometer (HEM-907 XL, Omron Corporation, Japan) before, and immediately after the running and arm exercising.

Post-training graded exercise tests in normoxia (Post-training testing 1 and 2)

After completing the HT or NT (post-training testing 1) and then also after completing HT or NT (post-training testing 2), athletes performed REx2 and then REx4, respectively, the following day in normoxic environment to evaluate the effect of HIIT and hypoxia on $\dot{V}O_2$peak, Ppeak, LT, $\dot{V}O_2$/LT, BLA, and biochemical variables. These tests were performed according to the study protocol presented in section 2.2.1.

Biochemical analyses

The blood samples were collected from the cubital vein at eight time points:

- REx1 (pre-training testing): At rest before and immediately after the test in normoxia (i.e. two blood withdrawals) and normobaric hypoxia (i.e. two blood withdrawals).
- REx2 (post-training testing: At rest before and immediately after the test, performed the day after completing HT (i.e. two blood withdrawals) or NT (i.e. two blood withdrawals).
- REx3 (pre-training testing): At rest before and immediately after the test in normoxia (i.e. two blood withdrawals) and normobaric hypoxia (i.e. two blood withdrawals).
- REx4 (post-training testing: At rest before and immediately after the test, performed the day after completing HT (i.e. two blood withdrawals) or NT (i.e. two blood withdrawals).

This sampling approach allowed for a comprehensive assessment of haematological and biochemical responses to HIIT in hypoxic and normoxic conditions.

To minimize the effects of diurnal variations, resting blood samples were collected in the morning during the same time period (between 8.00 and 9.00 am). Venous blood was withdrawn to evaluate the concentration of HIF-1α, VEGF, creatine kinase (CK), lactate dehydrogenase (LDH), troponin (TN), myoglobin (Mb), TGF-β, TNF-α, interleukin 6 (IL-6), and antioxidant enzymes (glutathione peroxidase, GPx; catalase, CAT; superoxide dismutase, SOD and glutathione reductase, GR). Capillary blood was collected from a fingertip for the determination of BLA at rest and throughout each stage of the exercise test to calculate the individual lactate threshold (LT).

The blood samples were left to clot at room temperature for 30 min and centrifuged for 15 min at 1000× g and kept frozen at -80°C (for a period not longer than 8 months) without repeated freezing. Red blood cells (RBC), white blood cells (WBC), and haemoglobin (Hb) levels were assessed in venous blood samples (anti-coagulated with EDTA) using an Automated Hematology Analyzer XS-1000*i*™ (Sysmex). The intra-assay coefficient of variation was < 4.4%, the inter-assay coefficient of variation was < 5.6% and the sensitivity was 0.5 pg/mL.

The measurements of BLA in a fingertip capillary blood were performed using the GEM Premier 3000 with IQM (Instrumentation Laboratory). CK and LDH were measured using the clinical chemistry analyzer SYNCHRON CX 9 PRO (Beckman Coulter) and a commercial kit (CK NAC and LDH P-L, RANDOX, UK). Intra-assay coefficients of variation for CK and LDH were 2.3 and 3.9, respectively. Serum (HIF)-1α was determined using an Enzyme-Linked Immunosorbent Assay ELISA Kit, BlueGene Biotech, China. Skeletal muscle TN was measured using a Human TNNI1 (Troponin I Type 1, Slow Skeletal ELISA Kit EH-0625, Fine Biological Technology, Co. Ltd. Wuhan, China), and serum Mb were assessed using a Human Myoglobin Enzyme Immunoassay (Myoglobin ELISA, KIT DRG® Myoglobin, EIA-3955). Intra-assay coefficients of variation for these assays were 3.9 and 7.8, respectively. Serum IL-6 was assessed by a Human IL-6 High Sensitivity ELISA Kit (Diaclone, France) and intra- and inter-assay variation coefficients for IL-6 were <4.4% and 4.6%, respectively. Serum TNF-α was assessed using Immuno Assays, DIAsource, Belgium. The intra- and inter-assay coefficient of variation for TNF-α was <4.6%, and <5.6%, respectively. Serum TGF-β and VEGF were determined by the Enzyme-Linked Immunosorbent Assay ELISA Kit, BlueGene Biotech, China. The intra-assay and inter-assay coefficients of variation for VEGF and TGF-β were <4.4% and <5.6%, respectively.

Heparinized blood samples were centrifuged for 10 min at 1000 x g at 4°C. Plasma and erythrocytes were separated and kept frozen at -80°C until analysis for activities of RBC antioxidant enzymes, i.e. GPx (EC 1.11.1.9), CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), and GR (EC 1.6.4.2). Blood samples were also assayed for GSH using the colorimetric method with 5,5′-dithiobis-2-nitrobenzoic acid. RBC antioxidant enzymes were analyzed using the method described by Sadowska-Krępa et al. (2017). Fresh whole blood samples were immediately assayed for reduced glutathione using the calorimetric method (Beutler et al., 1963). Changes in plasma volume were taken into account in the estimations. Biochemical analyses were performed in our certified laboratory, fulfilling the requirements of PN EN-ISO 9001:2009 (certificate no. 129/2015).

Statistical analysis

All analyses were performed using commercially available software (The Statistical Package v. 12; StatSoft Poland, 12.0). All normally distributed data are presented as means and standard deviations. The Shapiro–Wilk, the Levene's, and the Mauchly's tests were used to verify data normality, homogeneity, and sphericity, respectively. Verifications of the differences between analyzed variables (i.e., exercise training (pre- vs. post-training; T), oxygen conditions (normoxia vs. hypoxia; Ox), graded exercise test (rest vs. max; Ex) and interactions (Ox*T; Ox*Ex; T*Ex and Ox*T*Ex) were carried out using the repeated measures ANOVA. The partial eta squared (ηp^2) as the effect size were calculated. The results were interpreted on the basis of the large effect size. The criteria to interpret the magnitude of the effect sizes provided by Cohen (1988) were: small ($\eta p^2 \geq 0.01$, medium ($\eta p^2 \geq 0.06$), large ($\eta p^2 \geq 0.14$); in addition to ηp^2, Cohen's d for post-hoc analyses was separately calculated. For the Cohen's d, effect size was categorized as small (≥ 0.20), medium (≥ 0.50), and large (≥ 0.80). The significance of the differences between the variables was verified with the Bonferroni post hoc test; $p < 0.05$ indicated statistical significance.

Results

Participants' somatic characteristics

The participants' body mass and body height were 53.1 ± 6.4 kg and 164.1 ± 7.6 cm, respectively. Their body mass index (BMI) was 19.7 ± 1.8 kg/m², and body fat percentage (%FAT) was 13.4 ± 6.0%, both falling within the lower reference limits for this age group according to WHO guidelines (WHO, 2018).

Haematological variables and aerobic performance

A significant Ox*T interaction effect was observed for RBC count ($\eta p^2 = 0.398$, $p = 0.021$). Similarly, a significant effect of Ox conditions ($\eta p^2 = 0.736$, $p < 0.001$) and Ox*T interaction effect ($\eta p^2 = 0.689$, $p = 0.001$) were found for Hb levels. Post hoc comparisons revealed non-significant results for RBC (post - NT vs. post - HT; p = 0.312) and significantly higher Hb levels post - HT compared to pre - HT ($p < 0.05$) and compared to post - NT levels ($p < 0.05$).

A significant effect of Ox conditions ($\eta p^2 = 0.321$, $p = 0.044$) and of training ($\eta p^2 = 0.474$, $p = 0.009$) were found for $\dot{V}O_2$peak. A post hoc analysis revealed a significantly higher $\dot{V}O_2$peak ml/kg/min post-HT compared to the pre - HT ($p < 0.01$). For $\dot{V}O_2$peak a non-significant interaction effect (Ox*T) ($p = 0.090$) was found, however the partial eta squared ($\eta p^2 = 0.253$) indicated a large effect size. Moreover, there was a significant Ox*T interaction effect on $\dot{V}O_2$/LT ($\eta p^2 = 0.382$, $p = 0.024$). A significantly higher $\dot{V}O_2$/LT post - HT compared to post- NT ($p < 0.01$) and to pre-HT value ($p < 0.01$) was observed.

There was a significant effect of Ox conditions ($\eta p^2 = 0.527$, $p = 0.005$) and T ($\eta p^2 = 0.739$, $p = 0.000$) on Ppeak. The Ppeak increased significantly in response to HT

and NT (Table 1). An average workload (Watts) during HT vs. NT did not differ significantly. The relative training load for running exercise in hypoxia and normoxia was 11.8 ± 1.0 vs. 12.3 ± 0.9 km/h, respectively (p = 0.055), whilst the relative training load for arm cranking exercise under hypoxia and normoxia was 150.0 ± 34.0 vs. 155.8 ± 34.0 Watts (p = 0.05), respectively. Physiological variables recorded during the graded exercise tests before and after NT and HT are presented in Table 1.

Hypoxia-induced factors and *biomarkers of skeletal muscle damage*

Significant exercise effects were observed for hypoxia-induced factors ($p < 0.05$) and biomarkers of skeletal muscle damage (all p-values <0.01). However, no significant effects of Ox conditions or Ox*T interactions were found for HIF-1α concentrations. Oxygen conditions, as well as oxygen-by-training-by-exercise (Ox*T*Ex) interactions had a significant effect on VEGF concentrations ($\eta p^2 = 0.720$, $p < 0.001$; $\eta p^2 = 0.352$, $p = 0.033$, respectively). Post hoc comparisons revealed significantly higher VEGFmax (at maximal exercise intensity during REx2 or REx4) post-HT compared to pre-HT ($p < 0.05$).

For CK activity, a marginally non-significant interaction effect (Ox*T*Ex) ($p = 0.050$) was found, however the partial eta squared ($\eta p^2 = 0.310$) indicated a large effect size. There was also a non-significant interaction effect (Ox*T*Ex) on LDH level ($p = 0.232$) and a large effect size ($\eta p^2 = 0.127$). Post hoc analyses revealed significantly lower CKmax and LDHmax activity post - HT compared to post - NT ($p < 0.01$ and $p < 0.01$).

Significant interaction effects were observed for Mb concentrations in both the Ox*T interaction ($\eta p^2 = 0.617$, $p = 0.001$) and the Ox*T*Ex interaction ($\eta p^2 = 0.647$, $p = 0.001$). Post hoc analyses revealed significantly lower serum Mbmax post-HT compared to both pre-HT ($p < 0.05$) and post-NT ($p < 0.001$). No significant interaction effects were found for TN concentrations (Table 2).

Cytokines and oxidative status

A significant effect of Ox conditions ($\eta p^2 = 0.642$, $p = 0.001$), Ox*T ($\eta p^2 = 0.324$, $p = 0.042$) and Ox*Ex ($\eta p^2 = 0.575$, $p = 0.003$) was observed for IL-6 levels. Significantly lower IL-6max levels were observed post - HT compared to pre - HT ($p < 0.05$) and compared to post - NT ($p < 0.001$). The Ox*T interaction had a significant effect on the serum TNF-α ($\eta p^2 = 0.474$, $p = 0.009$) and TGF-β ($\eta p^2 = 0.410$, $p = 0.018$) (Table 3). A post-hoc analysis revealed significantly lower exercise-induced (max) TNF-α and TGF-β levels post-HT compared to post-NT ($p < 0.01$ and $p < 0.001$, respectively).

Table 1. Changes in baseline haematological parameters and physiological variables before (pre) and after (post) 3 weeks of exercise training in normoxia (NT) and hypoxia (HT).

	NT, n=12		HT, n=12		Main effects (p-value, ηp^2)		
Variables	***Pre***	***Post***	***Pre***	***Post***	**Ox**	**T**	**Ox*T**
RBC [10^6 /µL]	4.6 ± 0.3	4.5 ± 0.3	4.5 ± 0.3	4.7 ± 0.4	0.537 (0.036)	0.495 (0.043)	**0.021 (0.398)**
Hb [g/dL]	14.7 ± 1.4	13.7 ± 0.9	14.8 ± 1.0	15.5 ± 1.0*###	**0.000 (0.736)**	0.561 (0.032)	**0.001 (0.689)**
Hct [%]	38.6 ± 2.2	38.8 ± 2.2	38.3 ± 0.9	40.5 ± 2.9*	0.676 (0.017)	0.872 (0.003)	**0.001 (0.653)**
WBC [10^3/ µL]	4.0 ± 1.6	4.1 ± 0.9	3.8 ± 0.7	3.7 ± 0.8	0.527 (0.037)	0.499 (0.043)	0.282 (0.105)
$\dot{V}O_2$peak [mL/kg/min]	55.6 ± 9.9	56.6 ± 6.9	54.7 ± 8.8	58.5 ± 8.6**	**0.044 (0.321)**	**0.009 (0.474)**	0.079 (0.253)
Ppeak [Watt]	212.3 ± 10.3	233.0 ± 10.3^^	224.9 ± 10.8	243.0 ± 10.6***	**0.005 (0.527)**	**0.000 (0.739)**	0.446 (0.053)
LT [km/h]	11.8 ± 0.8	11.9 ± 1.0	11.8 ± 0.78	12.3 ± 0.9	0.477 (0.061)	0.166 (0.167)	0.104 (0.222)
$\dot{V}O_2$/LT [mL/kg/min]	44.9 ± 5.2	45.5 ± 7.0	45.0 ± 5.8	48.4 ± 4.8**##	**0.039 (0.334)**	**0.002 (0.581)**	**0.024 (0.382)**
BLA [mmoL/L]	7.7 ± 1.7	7.8 ± 1.3	7.8 ± 1.6	8.5 ± 1.8	0.284 (0.103)	0.068 (0.271)	0.109 (0.217)

RBC-red blood count; Hb-haemoglobin, Hct-haematocrit; WBC-white blood count, VO2peak - peak oxygen uptake; Ppeak- peak power; LT-lactate threshold; BLA-blood lactate; ox-oxygen conditions; T-training effects. Data are means and SD. *p < 0.05, ** p < 0.01 significant differences between post - HT vs. pre - HT; ^^p < 0.01 significant differences between post - NT vs. pre - NT; ##p < 0.01, ###p < 0.001 significant differences between post-HT vs. post-NT.

Table 2. Changes in hypoxia-induced markers and biomarkers of skeletal muscle before (pre) and after (post) 3 weeks of exercise training in normoxia (NT) and hypoxia (HT).

		NT, n=12		HT, n=12		Main effects (p-value, ηp^2)						
Variables		**Pre**	**Post**	**Pre**	**Post**	**Ox**	**T**	**Ex**	**Ox*T**	**Ox*Ex**	**T*Ex**	**Ox*T*Ex**
HIF-1α	rest	0.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.4	0.5 ± 0.3	0.359	0.778	0.005	0.162	0.351	0.654	0.050
[ng/mL]	max	0.8 ± 0.8	0.6 ± 0.3	0.6 ± 0.4	1.2 ± 0.5	(0.080)	(0.008)	(0.526)	(0.170)	(0.079)	(0.019)	(0.310)
VEGF	rest	41.5 ± 21.8	46.8 ± 32.1	42.5 ± 25.0	62.4 ± 25.0	0.359	0.778	0.005	0.162	0.351	0.654	0.050
[ng/mL]	max	51.5 ± 16.4	50.7 ± 22.5	60.1 ± 16.8	81.7 ± 16.8*	(0.080)	(0.008)	(0.526)	(0.170)	(0.079)	(0.019)	(0.310)
CK	rest	130.9 ± 61.2	134.5 ± 60.3	132.2 ± 34.0	125.9 ± 31.6	0.359	0.778	0.005	0.162	0.351	0.654	0.050
[U/L]	max	164.3 ± 77.4	177.3 ± 77.8	166.8 ± 57.4	145.7 ± 41.3##	(0.080)	(0.008)	(0.526)	(0.170)	(0.079)	(0.019)	(0.310)
LDH	rest	353.8 ± 49.8	361.2 ± 42.2	358.3 ± 33.9	334.1 ± 42.9	0.140	0.050	0.000	0.152	0.852	0.630	0.232
[U/L]	max	398.8 ± 54.0	411.6 ± 52.9	418 ± 50.7	375.5 ± 48.8**##	(0.187)	(0.041)	(0.844)	(0.177)	(0.003)	(0.022)	(0.127)
TN	rest	1.6 ± 0.5	1.6 ± 0.9	1.8 ± 1.2	2.0 ± 1.0	0.060	0.077	0.001	0.940	0.743	0.857	0.996
[ng/mL]	max	1.8 ± 1.0	2.0 ± 1.5	1.9 ± 1.4	2.4 ± 0.9	(0.286)	(0.257)	(0.647)	(0.001)	(0.010)	(0.003)	(0.000)
Mb	rest	38.4 ± 5.2	37.8 ± 7.2	38.5 ± 5.8	35.6 ± 7.6	0.083	0.625	0.000	0.001	0.062	0.414	0.001
[ng/mL]	max	42.7 ± 11.7	54.7 ± 11.8	47.2 ± 8.6	35.9 ± 12.2*###	(0.248)	(0.022)	(0.882)	(0.617)	(0.281)	(0.062)	(0.647)

HIF-1α - hypoxia-inducible factor 1 alpha; VEGF - vascular endothelial growth factor; CK - creatine kinase; LDH - lactate dehydrogenase; TN -troponin; Mb - myoglobin; rest – at baseline; max – immediately after graded exercise test. Ox-oxygen conditions; T-training and Ex-exercise effects. Data are means and SD. *p < 0.05, ** p < 0.01 significant differences between post HT vs. pre HT; #p < 0.05, ##p < 0.01, ###p < 0.001 significant differences between post-HT vs. post-NT.

Table 3. Changes in cytokines before (pre) and after (post) 3 weeks exercise training in normoxia (NT) and hypoxia (HT).

		NT, n=12		HT, n=12		Main effects (p-value, ηp^2)						
Variables		**Pre**	**Post**	**Pre**	**Post**	**Ox**	**T**	**Ex**	**Ox*T**	**Ox*Ex**	**T*Ex**	**Ox*T*Ex**
IL-6	rest	1.7 ± 0.6	1.5 ± 0.5	1.7 ± 0.4	1.2 ± 0.3	**0.001**	0.154	**0.000**	**0.042**	**0.048**	**0.003**	**0.051**
[pg/mL]	max	2.3 ± 0.3	2.6 ± 0.5	2.2 ± 0.7	1.7 ± 0.4*###	**(0.642)**	(0.175)	**(0.809)**	**(0.324)**	**(0.309)**	**(0.575)**	**(0.304)**
TNF-α	rest	46.2 ± 13.3	59.5 ± 15.9	51.6 ± 1.2	44.8 ± 15.8	**0.013**	0.338	**0.000**	**0.009**	0.833	0.313	0.577
[pg/mL]	max	65.0 ± 21.7	66.6 ± 21.5	69.1 ± 26.0	52.6 ± 21.2##	**(0.441)**	(0.084)	**(0.784)**	**(0.474)**	(0.248)	(0.092)	(0.029)
TGF-β	rest	442.3 ± 57.4	426.1 ± 49.2	442.3 ± 50.1	422.1 ± 34.4***###	0.097	**0.018**	**0.001**	**0.018**	**0.010**	0.142	**0.002**
[ng/mL]	max	504.6 ± 50.0	492.4 ± 51.8	500.5 ± 45.6	450.2 ± 38.2	(0.230)	**(0.410)**	**(0.641)**	**(0.410)**	**(0.469)**	(0.185)	**(0.410)**

IL-6HS-interleukin 6 highly sensitive, TNF-α-tumour necrosis factor alpha; TGF-β-transforming growth factor beta; rest – at baseline; max – immediately after graded exercise test. Ox-oxygen conditions; T-training and Ex-exercise effects. Data are means and SD. *p < 0.05, ***p < 0.001 significant differences between post-HT vs. pre-HT; ##p < 0.01, ###p < 0.001significant differences between post - HT vs. post - NT.

Table 4. Biomarkers of oxidative (pre) and after (post) 3 weeks exercise training in normoxia (NT) and hypoxia (HT).

		NT, n=12		HT, n=12		Main effects (p-value, ηp^2)						
Variables		**Pre**	**Post**	**Pre**	**Post**	**Ox**	**T**	**Ex**	**Ox*T**	**Ox*Ex**	**T*Ex**	**Ox*T*Ex**
SOD	rest	1248.2 ± 92.4	1215.1 ± 78.0	1250.0 ± 108.0	1152.5 ± 115.5	0.233	0.708	0.001	0.961	0.008	0.193	0.498
[U/gHb]	max	1195.2 ± 59.5	1195.0 ± 59.3	1195.0 ± 68.9	1250.6 ± 54.1	(0.126)	(0.031)	(0.615)	(0.000)	(0.484)	(0.148)	(0.043)
GPX	rest	47.0 ± 6.5	48.0 ± 8.5	48.1 ± 8.2	54.2 ± 6.1	0.008	0.034	0.000	0.104	0.175	0.501	0.147
[U/gHb]	max	54.7 ± 7.4	55.0 ± 9.4	55.7 ± 6.9	58.3 ± 8.6	(0.482)	(0.347)	(0.733)	(0.222)	(0.160)	(0.042)	(0.181)
CAT	rest	214.9 ± 38.5	219.6 ± 33.0	224.3 ± 27.9	234.5 ± 38.6	0.228	0.085	0.000	0.298	0.692	0.351	0.804
[U/gHb]	max	229.6 ± 18.6	232.5 ± 17.2	230.6 ± 15.4	235.8 ± 6.5	(0.129)	(0.246)	(0.785)	(0.098)	(0.015)	(0.079)	(0.006)
GSH	rest	2.9 ± 0.5	2.9 ± 0.7	2.9 ± 0.5	2.8 ± 0.5	0.365	0.221	0.020	0.025	0.010	0.366	0.092
[μg/mgHb]	max	3.0 ± 0.5	3.1 ± 0.5	3.2 ± 0.7	2.7 ± 0.4#	(0.075)	(0.133)	(0.404)	(0.379)	(0.468)	(0.075)	(0.237)

SOD-superoxide dismutase; GPX- glutathione peroxidase; CAT-catalase; GSH- reduced glutathione. Ox-oxygen conditions; T-training and Ex-exercise effects. Data are means and SD. #p < 0.05 significant differences between post - HT vs. post – NT.

ANOVA showed a significant effect of exercise on biomarkers of oxidative status ($p < 0.05$). As presented in Table 4, a significant Ox*E interaction effect was observed for SOD activity ($\eta p^2 = 0.484$, $p = 0.008$). Additionally, a significant effect of Ox conditions on GPX levels ($\eta p^2 = 0.482$, $p < 0.008$) was found. Significant Ox*T and Ox*Ex interaction effects were also observed for GSH levels ($\eta p^2 = 0.379$, $p = 0.025$; $\eta p^2 = 0.468$, $p = 0.010$, respectively). A post hoc analysis revealed significantly lower GSHmax levels post-HT compared to post - NT ($p < 0.05$). No significant Ox*T*Ex interaction effect was observed for any of the antioxidative status parameters (Table 4).

Discussion

The main observation in our study is that 3 weeks of high intensity interval training significantly increased aerobic performance and modified blood concentration of biomarkers of skeletal muscle fatigue in young biathletes. The changes in the biochemical variables after HIIT showed that hypoxia exhibited a protective effect against exercise-induced motor performance fatigue compared to normoxia. This was reflected in decreased levels of CK and Mb and pro-inflammatory cytokines (IL-6 and TNF-α).

Improvements in endurance performance in response to HT

The main observation in our study is that 3 weeks of HIIT in hypoxia significantly increased aerobic performance ($\dot{V}O_2$peak and Ppeak) compared to pre - hypoxia training value (main effect of oxygen conditions). The repeated measures of ANOVA show for $\dot{V}O_2$peak a non-significant interaction effect (Ox*T), however the partial eta squared indicated a large effect size. Importantly, reduction oxygen availability during exercise training resulted in a significant increase $\dot{V}O_2$LT during graded exercise tests compared to HIIT in normoxia. The significant improvement in aerobic performance in biathletes following hypoxia HIIT - an effect not observed in response to normoxia - is in line with findings suggesting that exercise training in hypoxia improves athletic performance to a greater extent than the same training regimen in normoxia (Czuba et al. 2019, Millet et al., 2010; Jung et al., 2020). Biathlon is a physically demanding sport in which cross-country skiing represents the most critical component of overall performance (Luchsinger et al., 2019; Björklund et al., 2022). High aerobic capacity, the ability to maintain high-intensity exercise and reduced susceptibility to both physical and mental fatigue are well-established determinants of overall competitive performance in elite biathletes (Rundell and Bacharach, 1995; Luchsinger et al., 2019; Köykkä et al., 2021; Björklund et al., 2022; Staunton et al., 2022).

Both HIIT and hypoxic training have been widely used to improve the performance of athletes (Brocherie et al. 2018; Vogt et al., 2001). The molecular responses triggered by stress induced by either high intensity exercise or hypoxia have been previously described (Li et al. 2020). However, whether high-altitude training increases aerobic fitness and improves recovery processes in young athletes is still debatable (Beidleman et al., 2009, Millet and Girard, 2017; Czuba et al., 2019). The physiological mechanisms responsible for these benefits include the improvements in the oxygen transport capacity through stimulating erythropoiesis and molecular mechanisms of oxygen utilization (Millet et al., 2010; Jung et al., 2020). However, these changes are dependent on altitude exposure, duration and time of exposure (Sinex and Chapman 2015; Park et al. 2022; Kasperska and Zembron-Lacny, 2020). Hypoxia exposure (12h per day for 4 weeks) induces the release of erythropoietin (EPO) (Camacho-Cardenosa et al., 2021). The exposure to hypoxia for 80 min per day, 3 days per week for 3 weeks, may be insufficient to significantly modify haematological variables (Beidleman et al., 2009; Sinex and Chapman, 2015). Therefore, longer

periods of hypoxic training (3 days per week for 6 weeks) are recommended to improve haematological parameters and endurance performance (Płoszczyca et al., 2018; Park et al., 2022). Conversely, a previous study reported that a short intermittent hypoxia protocol (4 – min bouts of intermittent hypoxia, representing duration of 32 min at an arterial oxygen saturation of 80%) has the potential to increase serum EPO levels in healthy individuals (Wojan et al. 2021).

Our results are consistent with the previously published data demonstrating that a 3-week HIIT in hypoxia leads to a significant increase in Hb and Hct levels compared to pre - HT and post - NT levels (Żebrowska et al. 2019). In the present study, we observed a significant oxygen-by-training interaction effect for RBC count. However, no significant changes in RBC count were found in response to HT. This may suggest that the observed increase in Hb and Hct following HT could potentially be attributed to reductions in plasma volume via increased diuresis rather than to erythropoiesis *per se* (Swenson et al., 1985). It has been previously shown that exposure to hypoxia stimulates diuresis, thereby increasing blood oxygen-carrying capacity (Swenson et al., 1985; Roche et al. 2022) before hypoxia-induced erythropoiesis could become effective. However, the positive effects of hypoxia on aerobic performance could also be attributed to an optimal increase in Hct, resulting in a favorable balance between haematological parameters and plasma volume (Sitina et al., 2021). It seems reasonable to assume that a longer duration of HT (i.e. > 3 weeks) may lead to more pronounced haematological adaptations in young athletes.

In our study, HT also resulted in a significant improvement in $\dot{V}O_2$LT and Ppeak during graded exercise tests compared to the pre-HT values. Laaksonen et al. (2020) have previously demonstrated that $\dot{V}O_2$ at LT is an important determinant of skiing performance. Our findings support previous observations and confirm that both HIIT and hypoxia are effective strategies for enhancing submaximal and maximal performance. The potential mechanism underlying this effect may be associated with hypoxia-induced activation of cellular mechanisms responsible for oxygen sensing and enhanced oxygen delivery and utilization within the working muscles (Vogt et al., 2001; Burtscher et al. 2024, Favier et al., 2015; Jung et al., 2020). One factor that may contribute to this phenomenon is HIF-1α, which mediates the expression of genes encoding glucose transporters and glycolytic enzymes (Chen and Gaber, 2021; Abe et al., 2015), as well as genes encoding angiogenic factors such as VEGF (Liu et al. 1995; Favier et al., 2015). It has been previously shown that three days of hypoxic training per week for 4 weeks with an intensity of 100% $\dot{V}O_2$max is associated with higher HIF-1α and plasma VEGF concentrations compared to normoxic training (Favier et al., 2015). These adaptations may promote angiogenesis and, consequently, contribute to improvements in aerobic performance. In our study, HIIT in hypoxia led to elevated post exercise VEGF serum levels (effect of Ex). A significant effect of both Ox conditions and training was observed for VEGF, whereas no significant changes were found in HIF-1α serum levels. These results are consistent with previous research demonstrating that hypoxic training increases the expression of VEGF (Breen et al., 2008) but not of the muscle HIF-1α (De Smet et al., 2018). The increase in VEGF during hypoxia is primarily mediated via HIF-1 pathway, although several other mechanisms have been reported (Breen et al. 2008). These include the production of oxygen related protein 150 (ORP150), adenylate/uridylate-rich elements (AREs), insulin growth factor or nitric oxide signaling pathways. Additionally, HIIT performed under hypoxic conditions has been shown to stimulate mitochondrial biogenesis, as evidenced by increased mitochondrial mass and the upregulation of genes involved in mitochondrial function, including nuclear respiratory factors (NRFs) and mitochondrial transcription factor A (TFAM). These changes are primarily regulated by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which acts as a master regulator of mitochondrial biogenesis, together with other key molecular mediators such as VEGF and HIF-1α, driving skeletal muscle adaptation to HIIT in hypoxia (Breen et al., 2008; Faiss et al., 2013; Chacaroun et al., 2017; Żebrowska et al., 2019).

Previous studies have suggested that differences in physiological adaptations to exercise in hypoxic conditions may be attributed to the relatively lower exercise intensity in hypoxia compared to normoxia (Czuba et al. 2019). In the present study, however, the intensity of the training sessions (exceeding 75% of the Ppeak) was sufficient to induce a high training load (Li et al. 2020). Moreover, the relative training load was comparable between hypoxic and normoxic conditions, with no significant differences observed between running exercise in normoxia vs hypoxia (p = 0.055) and arm cranking exercise in normoxia vs hypoxia (p = 0.05) in biathletes. We believe that HT protocol used in this study was effective in enhancing biathletes' overall athletic performance, and that the positive outcomes observed may be attributed to individual physiological responses to high intensity interval workouts performed in hypoxia.

Hypoxia-induced factors and motor performance fatigue

Considering that intensive exercise training may induce muscle fatigue characterized by a decreased motor performance and an increased perception of fatigue in youth athletes (Vinet et al., 2003), we aimed to determine whether training in hypoxia of the same intensity as in normoxia, may have a protective effect, reflected by lower serum concentrations of biomarkers of skeletal muscle fatigue and inflammation (Gangwar et al., 2020; Hill et al., 2020; Sumi et al., 2021). It has been demonstrated that biomarkers of skeletal muscle fatigue can provide insights into the mechanisms of exhaustion during exercise, including: (1) metabolic acidosis, indicated by elevated serum lactate and ammonia levels, (2) overproduction of ROS, reflected by markers of lipid peroxidation and changes in antioxidant capacity and (3) local inflammatory reactions indicated by increased TNF-α, interleukins and skeletal muscle damage markers (CK, TN and Mb) (Finsterer, 2012, Haller et al. 2023, Peake et al., 2017).

In our study, a significant effect of the HT on Mb serum and a marginally insignificant (p =0.05) effect of HT

on CK serum levels were found. Here it is worth mentioning that the partial eta squared indicated a *large* effect size for CK, which could suggest a meaningful physiological impact of HT on muscle damage reduction, even if it did not reach statistical significance. This finding may reflect individual variability in the response to HT or a limited sample size and hence warrants further investigation. Both Mb and CK significantly decreased in response to HT, which was not observed in response to NT. We did not observe any effects of HT on the levels of TN.

The effects of hypoxia exposure and training on biomarkers of skeletal muscle damage have been previously reported (Abe et al., 2015; Chacaroun et al., 2017; De Groote et al., 2018). Strenuous exercise transiently reduces muscle power and elevates serum levels of Mb, a haemoprotein found in the oxidative skeletal and cardiac myocytes. It exhibits a high affinity for oxygen from blood Hb. The Mb's oxygen affinity and oxygen delivery to the tissues are important adaptive mechanisms to hypoxic conditions (Endeward, 2012). In previous studies, endurance exercise in hypoxia significantly decreased Mb serum concentrations compared to normoxic endurance exercise (Sumi et al., 2018; 2021), however, did not significantly affect the skeletal muscles' Mb (Masuda et al., 2001). Other studies demonstrated that Mb and CK activity increased within the normal range (e.g., 0 to 0.003 mg/dl and 22 to −198 U/l, respectively) due to muscle cells damage and changes in the muscle cell membrane permeability (Mougios, 2007; Lee et al., 2017). We observed decreased muscle damage in hypoxic versus normoxic conditions, which has also been shown elsewhere (Sumi et al., 2018). Interestingly, we found no significant effect of HT on WBC and neutrophil counts. Although, there was a significant effect of hypoxia on the pro-inflammatory cytokines IL-6 and TNF-α. Significantly lower levels of these cytokines were observed in response to the HT compared to NT.

Cytokines and oxidative status

Exposure to hypoxia modulates cytokines secretion and anti-inflammatory processes following exercise (Lira et al., 2017; Britto et al., 2020). Hypoxia has been shown to increase the secretion of pro-inflammatory cytokines and lead to excessive ROS production (Sánchez-Elsner et al., 2001; Pialoux et al., 2009; Goods et al., 2016; Turner et al., 2017; Morrison et al., 2018). Additionally, when combined with endurance training, hypoxia causes a greater IL-6 elevation (Chen and Gaber, 2021), likely due to the additive effects of hypoxia and exercise training on metabolic stress (Nash et al. 2023). In our study, we observed significantly lower levels of IL-6, TNF-α and TGF-β in response to HT compared to NT. The reduced TGF-β suggests that HT did not induce excessive ROS production (Liu and Desai 2015), while the significant reduction in IL-6 and TNF-α may indicate an adaptive response to HIIT and hypoxia (Petersen and Pedersen, 2005; Pedersen and Steensberg, 2002). Furthermore, the lower concentrations of these cytokines may be attributed to reduced activities of the redox-sensitive intracellular signalling pathways, which could blunt the cytokines release (Hill et al., 2020). Also, we only assessed cytokine levels before and immediately after the exercise training in hypoxic and normoxic conditions to minimise repeated blood sampling in our young participants. A single measurement of exercise-induced cytokines does not allow for a precise determination of chronic inflammation or anti-inflammatory effects of exercise training in athletes (Costache et al., 2021). Therefore, to better understand these responses, cytokines and other endocrine markers should be measured over a longer post-exercise recovery period (Lee et al., 2017; Hill et al. 2020).

Hypoxic training may disrupt the balance between pro- and antioxidant status via excessive production of ROS, which damage the cell membranes, enzymes and cause lipid peroxidation, negatively affecting athletic performance and health (Bailey et al. 2001). One of the adaptive responses to increased ROS release includes the upregulation of endogenous antioxidant enzymes, such as SOD, GPX, and CAT (Tsukiyama et al., 2017), which help to counteract oxidative stress and maintain cellular homeostasis. In our study, the 3 week HIIT in normobaric hypoxia was not a sufficient stimulus to increase the total antioxidant defence system (Goods et al., 2016; Simioni et al., 2018). However, Ox conditions had a significant effect on GPX, while the combination of Ox and training affected GSH levels. Although, we did not measure ROS concentrations in response to HT (and NT), we speculate that their production was not excessive as it did not lead to the upregulation of antioxidant enzymes. Notably, the antioxidant effect on HT was demonstrated through a reduction in TNF-α levels, suggesting that blocking TNF-α signalling suppressed pro-oxidant activity (Faiss et al., 2013; Favier et al., 2015; Turner et al., 2017; Chen and Gaber, 2021).

Limitations

This study has several limitations. Firstly, the relatively small size of the studied group did not allow us to examine the HT's effects in females and males. Nevertheless, our study participants were all elite biathletes from the same division of Polish Biathlon Association and underwent the same training regimen in hypoxic and normoxic conditions. Secondly, blood volume changes during exercise in hypoxia and normoxia should be considered when comparing the effects of fluid shifts on hemodynamic responses. To determine whether the observed reduction in the muscle damage biomarkers level and the associated and maintenance of athletic performance is possible further investigation is needed. Specifically, haematological responses, mitochondrial adaptations, and haemoconcentration effects should be studied over an extended period following hypoxic training. Despite this, biochemical variables measured at baseline and in response to HT/NT provided valuable information related to biathletes' performance and peripheral muscle fatigue. Also, we propose that the biomarkers of muscle fatigue and VEGF can serve as indicators of sport training effectiveness in young biathletes.

Conclusion

Our study demonstrates that three weeks of high intensity interval training in normobaric hypoxia elicits beneficial

effects as evidenced by improved aerobic performance in young biathletes. The training effectiveness may be attributed to two key mechanisms: enhanced skeletal muscle adaptations to submaximal and high-intensity exercise and decreased serum levels of biomarkers of skeletal muscle damage. However, further studies with extending the duration of hypoxic training and biomarkers monitoring are needed to confirm the efficacy of HIIT in hypoxia in young biathletes.

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References

Abe, T., Kitaoka, Y., Kikuchi, D. M., Takeda, K., Numata, O. and Takemasa, T. (2015) High-intensity interval training-induced metabolic adaptation coupled with an increase in Hif-1α and glycolytic protein expression. *Journal of Applied Physiology* **119**, 1297-1302. https://doi.org/10.1152/japplphysiol.00499.2015

Armstrong, N. and Welsman, J. (2019) Multilevel allometric modelling of maximal stroke volume and peak oxygen uptake in 11-13-year-olds. *European Journal of Applied Physiology* **119**, 2629-2639. https://doi.org/10.1007/s00421-019-04241-3

Bailey, D. M., Davies, B. and Young, I. S. (2001) Intermittent hypoxic training: implications for lipid peroxidation induced by acute normoxic exercise in active men. *Clinical Science* **101(5)**, 465-475. https://doi.org/10.1042/cs1010465

Baranauskas, M. N., Fulton, T. J., Fly, A. D., et al. (2022) High intraindividual variability in the response of serum erythropoietin to multiple simulated altitude exposures. *High Altitude Medicine and Biology* **23**, 85-89. https://doi.org/10.1089/ham.2021.0154

Behrens, M., Gube, M., Chaabene, H., et al. (2023) Fatigue and human performance: an updated framework. *Sports Medicine* **53**, 7-31. https://doi.org/10.1007/s40279-022-01748-2

Beidleman, B. A., Muza, S. R., Fulco, C. S., Jones, J. E., Lammi, E., Staab, J. E., et al. (2009) Intermittent hypoxic exposure does not improve endurance performance at altitude. *Medicine and Science in Sports and Exercise* **41**, 1317-1325. https://doi.org/10.1249/MSS.0b013e3181954601

Beutler, E., Duron, O. and Kelly, B. M. (1963) Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine* **61**, 882-888.

Björklund, G., Dzhilkibaeva, N., Gallagher, C. and Laaksonen, M. S. (2022) The balancing act between skiing and shooting - the determinants of success in biathlon pursuit and mass start events. *Journal of Sports Sciences* **40**, 96-103. https://doi.org/10.1080/02640414.2021.1976493

Björklund, G. and Laaksonen, M. S. (2022) The determinants of performance in Biathlon World Cup sprint and individual competitions. *Frontiers in Sports and Active Living* **4**, 841619. https://doi.org/10.3389/fspor.2022.841619

Breen, E., Tang, K., Olfert, M., Knapp, A. and Wagner, P. (2008) Skeletal muscle capillarity during hypoxia: VEGF and its activation. *High Altitude Medicine and Biology* **9**, 158-166. https://doi.org/10.1089/ham.2008.1010

Britto, F. A., Gnimassou, O., De Groote, E., Balan, E., Warnier, G., Everard, A., et al. (2020) Acute environmental hypoxia potentiates satellite cell-dependent myogenesis in response to resistance exercise through the inflammation pathway in humans. *FASEB Journal* **34**, 1885-1900. https://doi.org/10.1096/fj.201902244R

Brocherie, F., Girard, O., Faiss, R. and Millet, G. P. (2017) Effects of repeated-sprint training in hypoxia on sea-level performance: A meta-analysis. *Sports Medicine* **47**, 1651-1660. https://doi.org/10.1007/s40279-017-0685-3

Brocherie, F., Millet, G. P., D'Hulst, G., Van Thienen, R., Deldicque, L. and Girard, O. (2018) Repeated maximal-intensity hypoxic exercise superimposed to hypoxic residence boosts skeletal muscle transcriptional responses in elite team-sport athletes. *Acta Physiologica* **222**, e12851. https://doi.org/10.1111/apha.12851

Buckley, S., Knapp, K., Lackie, A., Lewry, C., Horvey, K., Benko, C., et al. (2015) Multimodal high-intensity interval training increases muscle function and metabolic performance in females. *Applied Physiology Nutrition and Metabolism* **40**, 1157-1162. https://doi.org/10.1139/apnm-2015-0238

Burtscher, J., Citherlet, T., Camacho-Cardenosa, A., Camacho-Cardenosa, M., Raberin, A., Krumm, B., et al. (2024) Mechanisms underlying the health benefits of intermittent hypoxia conditioning. *Journal of Physiology* **602(21)**, 5757-5783. https://doi.org/10.1113/JP285230

Burtscher, M., Millet, G. P. and Burtscher, J. (2022) Hypoxia conditioning for high-altitude pre-acclimatization. *Journal of Science in Sport and Exercise* **4**, 331-345. https://doi.org/10.1007/s42978-021-00150-0

Camacho-Cardenosa, M., Camacho-Cardenosa, A., Kemp, J., Brazo-Sayavera, J., Timon, R. and Olcina, G. (2021) Haematological responses to repeated sprints in hypoxia across different sporting modalities. *Research in Sports Medicine*, 1-11. https://doi.org/10.1080/15438627.2021.1917403

Chacaroun, S., Borowik, A., Morrison, S. A., Baillieul, S., Flore, P., Doutreleau, S. and Bricout, V. (2017) Physiological responses to two hypoxic conditioning strategies in healthy subjects. *Frontiers in Physiology* **7**, 675. https://doi.org/10.3389/fphys.2016.00675

Chen, Y. and Gaber, T. (2021) Hypoxia/HIF modulates immune responses. *Biomedicines* **9**, 260. https://doi.org/10.3390/biomedicines9030260

Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A. and Hesselink, M. (1992) A new approach for the determination of ventilatory and lactate thresholds. *International Journal of Sports Medicine* **13(7)**, 518-522. https://doi.org/10.1055/s-2007-1021309

Coates, A. M., Joyner, M. J., Little, J. P., et al. (2023) A perspective on high-intensity interval training for performance and health. *Sports Medicine* **53(Suppl 1)**, 85-96. https://doi.org/10.1007/s40279-023-01938-6

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Lawrence Erlbaum Associates.

Costache, A.-D., Costache, I.-I., Miftode, R.-Ș., Stafie, C.-S., Leon-Constantin, M.-M., Roca, M. and Petrisor, B. (2021) Beyond the finish line: The impact and dynamics of biomarkers in physical exercise. A narrative review. *Journal of Clinical Medicine* **10**, 4978. https://doi.org/10.3390/jcm10214978

Czarkowska-Paczek, B., Bartlomiejczyk, I. and Przybylski, J. (2006) The serum levels of growth factors: PDGF, TGF-beta and VEGF are increased after strenuous physical exercise. *Journal of Physiology and Pharmacology* **57(2)**, 189-197.

Czuba, M., Bril, G., Płoszczyca, K., Piotrowicz, Z., Chalimoniuk, M., Roczniok, R. and Wilk, M. (2019) Intermittent hypoxic training at lactate threshold intensity improves aiming performance in well-trained biathletes with little change of cardiovascular variables. *Biomedical Research International* **2019**, 1287506. https://doi.org/10.1155/2019/1287506

De Groote, E., Britto, F. A., Bullock, L., François, M., de Buck, C., Nielens, H., et al. (2018) Hypoxic training improves normoxic glucose tolerance in adolescents with obesity. *Medicine and Science in Sports and Exercise* **50**, 2200-2208. https://doi.org/10.1249/MSS.0000000000001694

De Smet, S., D'Hulst, G., Poffé, C., Van Thienen, R., Berardi, E. and Hespel, P. (2018) High-intensity interval training in hypoxia does not affect muscle HIF responses to acute hypoxia in humans. *European Journal of Applied Physiology* **118**, 847-862. https://doi.org/10.1007/s00421-018-3820-4

Dotan, R. (2017) Children's aerobic trainability and related questions. *Research Quarterly for Exercise and Sport* **88**, 377-383. https://doi.org/10.1080/02701367.2017.1371546

Endeward, V. (2012) The rate of the deoxygenation reaction limits myoglobin- and hemoglobin-facilitated O_2 diffusion in cells.

Journal of Applied Physiology **112**, 1466-1473. https://doi.org/10.1152/japplphysiol.00835.2011

Faiss, R., Pialoux, V., Sartori, C., Faes, C., Dériaz, O. and Millet, G. P. (2013) Ventilation, oxidative stress, and nitric oxide in hypobaric versus normobaric hypoxia. *Medicine and Science in Sports and Exercise* **45**, 253-260. https://doi.org/10.1249/MSS.0b013e31826d5aa2

Favier, F. B., Britto, F. A., Freyssenet, D. G., Bigard, X. A. and Benoit, H. (2015) HIF-1-driven skeletal muscle adaptations to chronic hypoxia: Molecular insights into muscle physiology. *Cellular and Molecular Life Sciences* **72**, 4681-4696. https://doi.org/10.1007/s00018-015-2025-9

Finsterer, J. (2012) Biomarkers of peripheral muscle fatigue during exercise. *BMC Musculoskeletal Disorders* **13**, 218. https://doi.org/10.1186/1471-2474-13-218

Fitzpatrick, J. and Panagodage Perera, N. (2020) The Biathlon Injury and Illness Surveillance (BIIS) project protocol: A prospective cohort study across two World Cup seasons. *BMJ Open Sport and Exercise Medicine* **6**, e000862. https://doi.org/10.1136/bmjsem-2020-000862

Gangwar, A., Paul, S., Ahmad, Y. and Bhargava, K. (2020) Intermittent hypoxia modulates redox homeostasis, lipid metabolism associated inflammatory processes and redox post-translational modifications: Benefits at high altitude. *Scientific Reports* **10**, 7899. https://doi.org/10.1038/s41598-020-64848-x

Goods, P. S. R., Dawson, B., Landers, G. J., Gore, C. J., Croft, K. and Peeling, P. (2016) Effect of repeat-sprint training in hypoxia on post-exercise interleukin-6 and F2-isoprostanes. *European Journal of Sport Science* **16**, 1047-1054. https://doi.org/10.1080/17461391.2015.1123776

Haller, N., Behringer, M., Reichel, T., et al. (2023) Blood-based biomarkers for managing workload in athletes: Considerations and recommendations for evidence-based use of established biomarkers. *Sports Medicine* **53**, 1315-1333. https://doi.org/10.1007/s40279-023-01836-x

Hering, S., Jost, C., Schulz, H., Hellmich, B., Schatz, H. and Pfeiffer, A. (2002) Circulating transforming growth factor β1 (TGFβ1) is elevated by extensive exercise. *European Journal of Applied Physiology* **86**, 406-410. https://doi.org/10.1007/s00421-001-0537-5

Hill, G. W., Gillum, T. L., Lee, B. J., Romano, P. A., Schall, Z. J., Hamilton, A. M., et al. (2020) Prolonged treadmill running in normobaric hypoxia causes gastrointestinal barrier permeability and elevates circulating levels of pro- and anti-inflammatory cytokines. *Applied Physiology Nutrition and Metabolism* **45**, 376-386. https://doi.org/10.1139/apnm-2019-0378

Hoier, B., Passos, M., Bangsbo, J. and Hellsten, Y. (2013) Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Experimental Physiology* **98**, 585-597. https://doi.org/10.1113/expphysiol.2012.067967

International Biathlon Union - IBU Datacenter. (2019) Datacenter (IBU). Available at: http://biathlonresults.com/ [Accessed November 16, 2020]

Jarrard, C. P., Nagel, M. J., Stray-Gundersen, S., Tanaka, H. and Lalande, S. (2021) Hypoxic preconditioning attenuates ischemia-reperfusion injury in young healthy adults. *Journal of Applied Physiology* **130**, 846-852. https://doi.org/10.1152/japplphysiol.00772.2020

Jonsson Kårström, M., McGawley, K. and Laaksonen, M. S. (2019) Physiological responses to rifle carriage during roller-skiing in elite biathletes. *Frontiers in Physiology* **10**, 1519. https://doi.org/10.3389/fphys.2019.01519

Jung, W.-S., Kim, S.-W. and Park, H.-Y. (2020) Interval hypoxic training enhances athletic performance and does not adversely affect immune function in middle- and long-distance runners. *International Journal of Environmental Research and Public Health* **17**, 1934. https://doi.org/10.3390/ijerph17061934

Kasperska, A. and Zembron-Lacny, A. (2020) The effect of intermittent hypoxic exposure on erythropoietic response and hematological variables in elite athletes. *Physiological Research* **69**, 283-290. https://doi.org/10.33549/physiolres.934316

Köykkä, M., Ihalainen, S., Linnamo, V., Ruotsalainen, K., Häkkinen, K. and Laaksonen, M. S. (2021) Aiming strategy affects performance-related factors in biathlon standing shooting. *Scandinavian Journal of Medicine and Science in Sports* **31**, 573-585. https://doi.org/10.1111/sms.13864

Laaksonen, M. S., Andersson, E., Jonsson Kårström, M., Lindblom, H. and McGawley, K. (2020) Laboratory-based factors predicting skiing performance in female and male biathletes. *Frontiers in Sports and Active Living* **2**, 99. https://doi.org/10.3389/fspor.2020.00099

Lee, E. C., Fragala, M. S., Kavouras, S. A., Queen, R. M., Pryor, J. L. and Casa, D. J. (2017) Biomarkers in sports and exercise: Tracking health, performance, and recovery in athletes. *Journal of Strength and Conditioning Research* **31**, 2920-2937. https://doi.org/10.1519/JSC.0000000000002122

Li, J., Li, Y., Atakan, M. M., Kuang, J., Hu, Y., Bishop, D. J. and Yan, X. (2020) The molecular adaptive responses of skeletal muscle to high-intensity exercise/training and hypoxia. *Antioxidants* **9(8)**, 656. https://doi.org/10.3390/antiox9080656

Lira, F. S., Lemos, V. A., Bittar, I. G., Caris, A. V., Dos Santos, R. V., Tufik, S., et al. (2017) Physiological and cytokine response to acute exercise under hypoxic conditions: A pilot study. *Journal of Sports Medicine and Physical Fitness* **57**, 461-468. https://doi.org/10.23736/S0022-4707.16.06073-X

Liu, Y., Cox, S. R., Morita, T. and Kourembanas, S. (1995) Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. *Circulation Research* **77(3)**, 638-643. https://doi.org/10.1161/01.res.77.3.638

Liu, R. M. and Desai, L. P. (2015) Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biology* **6**, 565-577. https://doi.org/10.1016/j.redox.2015.09.009

Luchsinger, H., Kocbach, J., Ettema, G. and Sandbakk, Ø. (2019) The contribution from cross-country skiing and shooting variables on performance-level and sex differences in biathlon World Cup individual races. *International Journal of Sports Physiology and Performance* **14**, 190-195. https://doi.org/10.1123/ijspp.2018-0134

Masuda, K., Okazaki, K., Kuno, S., Asano, K., Shimojo, H. and Katsuta, S. (2001) Endurance training under 2500-m hypoxia does not increase myoglobin content in human skeletal muscle. *Medicine and Science in Sports and Exercise* **33**, 2036-2042. https://doi.org/10.1097/00005768-200112000-00014

Millet, G. P. and Girard, O. (2017) Editorial: High-intensity exercise in hypoxia: Beneficial aspects and potential drawbacks. *Frontiers in Physiology* **8**, 1017. https://doi.org/10.3389/fphys.2017.01017

Millet, G. P., Roels, B., Schmitt, L., Woorons, X. and Richalet, J. P. (2010) Combining hypoxic methods for peak performance. *Sports Medicine* **40**, 1-25. https://doi.org/10.2165/11317920-000000000-00000

Morrison, J., Larsen, B., Cox, A. J. and Minahan, C. (2018) The post-exercise inflammatory response to repeated-sprint running in hypoxia. *Journal of Sports Science and Medicine* **17**, 533-538. https://pubmed.ncbi.nlm.nih.gov/30479520/

Mougios, V. (2007) Reference intervals for serum creatine kinase in athletes. *British Journal of Sports Medicine* **41**, 674-678. https://doi.org/10.1136/bjsm.2006.034041.

Nash, D., Hughes, M. G., Butcher, L., Aicheler, R., Smith, P., Cullen, T. and Webb, R. (2023) IL-6 signaling in acute exercise and chronic training: Potential consequences for health and athletic performance. *Scandinavian Journal of Medicine and Science in Sports*, 4-19. https://doi.org/10.1111/sms.14241.

Park, H.-Y., Jung, W.-S., Kim, S.-W., Kim, J. and Lim, K. (2022) Effects of interval training under hypoxia on hematological parameters, hemodynamic function, and endurance exercise performance in amateur female runners in Korea. *Frontiers in Physiology* **13**, 919008. https://doi.org/10.3389/fphys.2022.919008

Peake, J. M., Neubauer, O., Della Gatta, P. A. and Nosaka, K. (2017) Muscle damage and inflammation during recovery from exercise. *Journal of Applied Physiology* **122**, 559-570. https://doi.org/10.1152/japplphysiol.00971.2016

Pedersen, B. K. and Steensberg, A. (2002) Exercise and hypoxia: Effects on leukocytes and interleukin-6 - shared mechanisms? *Medicine and Science in Sports and Exercise* **34**, 90-98. https://doi.org/10.1097/00005768-200212000-00022

Petersen, A. M. and Pedersen, B. K. (2005) The anti-inflammatory effect of exercise. *Journal of Applied Physiology* **98(4)**, 1154-1162. https://doi.org/10.1152/japplphysiol.00164.2004

Pialoux, V., Brugniaux, J. V., Fellmann, N., Richalet, J.-P., Robach, P., Schmitt, L. et al. (2009) Oxidative stress and HIF-1 alpha modulate hypoxic ventilatory responses after hypoxic training on athletes. *Respiratory Physiology and Neurobiology* **167**, 217-

220. https://doi.org/10.1016/j.resp.2009.04.012
Płoszczyca, K., Langfort, J. and Czuba, M. (2018) The effects of altitude training on erythropoietic response and hematological variables in adult athletes: A narrative review. *Frontiers in Physiology* **9**, 375. https://doi.org/10.3389/fphys.2018.00375
Roche, J., Rasmussen, P., Gatterer, H., Roveri, G., Turner, R., van Hall, G., Maillard, M., Walzl, A., Kob, M., Strapazzon, G., Goetze, J. P., Schäfer, S. T., Kammerer, T., Nader, E., Connes, P., Robert, M., Mueller, T., Feraille, E. and Siebenmann, C. (2022) Hypoxia briefly increases diuresis but reduces plasma volume by fluid redistribution in women. *American Journal of Physiology. Heart and Circulatory Physiology* **323(6)**, H1068-H1079. https://doi.org/10.1152/ajpheart.00394.2022
Rundell, K. W. and Bacharach, D. W. (1995) Physiological characteristics and performance of top U.S. biathletes. *Medicine and Science in Sports and Exercise* **27**, 1302-1310. https://doi.org/10.1249/00005768-199509000-00010
Sadowska-Krępa, E., Kłapcińska, B., Pokora, I., Domaszewski, P., Kempa, K. and Podgórski, T. (2017) Effects of six-week Ginkgo biloba supplementation on aerobic performance, blood pro/antioxidant balance, and serum brain-derived neurotrophic factor in physically active men. *Nutrients* **9**, E803. https://doi.org/10.3390/nu9080803
Safdar, A., Little, J. P., Stokl, A. J., Hettinga, B. P., Akhtar, M. and Tarnopolsky, M. A. (2018) Exercise increases mitochondrial PGC-1 alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *Journal of Biological Chemistry* **293**, 4953. https://doi.org/10.1074/jbc.EC118.002682
Semenza, G. L. and Wang, G. L. (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Molecular and Cellular Biology* **12**, 5447-5454. https://doi.org/10.1128/MCB.12.12.5447
Sánchez-Elsner, T., Botella, L. M., Velasco, B., Corbí, A., Attisano, L. and Bernabéu, C. (2001) Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. *Journal of Biological Chemistry* **276**, 38527-38535. https://doi.org/10.1074/jbc.M104536200
Sandbakk, Ø., Sandbakk, S. B., Ettema, G. and Welde, B. (2013) Effects of intensity and duration in aerobic high-intensity interval training in highly trained junior cross-country skiers. *Journal of Strength and Conditioning Research* **27**, 1974-1980. https://doi.org/10.1519/JSC.0b013e3182752f08
Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A. and Neri, L. M. (2018) Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget* **9(24)**, 17181-17198. https://doi.org/10.18632/oncotarget.24729
Sinex, J. and Chapman, R. (2015) Hypoxic training methods for improving endurance exercise performance. *Journal of Sport and Health Science* **4**. https://doi.org/10.1016/j.jshs.2015.07.005
Sitina, M., Stark, H. and Schuster, S. (2021) Calculating the optimal hematocrit under the constraint of constant cardiac power. *Scientific Reports* **11(1)**, 3925. https://doi.org/10.1038/s41598-021-83427-2
Staunton, C. A., Sloof, L., Brandts, M., Jonsson Kårström, M., Laaksonen, M. S. and Björklund, G. (2022) The effect of rifle carriage on the physiological and accelerometer responses during biathlon skiing. *Frontiers in Sports and Active Living* **4**, 813784. https://doi.org/10.3389/fspor.2022.813784
Sumi, D., Kojima, C. and Goto, K. (2018) Impact of endurance exercise in hypoxia on muscle damage, inflammatory and performance responses. *Journal of Strength and Conditioning Research* **32**, 1053-1062. https://doi.org/10.1519/JSC.0000000000001911
Sumi, D., Yamaguchi, K. and Goto, K. (2021) Impact of three consecutive days of endurance training under hypoxia on muscle damage and inflammatory responses. *Frontiers in Sports and Active Living* **3**, 663095. https://doi.org/10.3389/fspor.2021.663095
Swenson, E. R., Duncan, T. B., Goldberg, S. V., Ramirez, G., Ahmad, S. and Schoene, R. B. (1985) Diuretic effect of acute hypoxia in humans: relationship to hypoxic ventilatory responsiveness and renal hormones. *Journal of Applied Physiology* **78(2)**, 377-383. https://doi.org/10.1152/jappl.1995.78.2.377
Tilg, H., Dinarello, C. A. and Mier, J. W. (1997) IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunology Today* **18**, 428-432. https://doi.org/10.1016/S0167-5699(97)01103-1
Tobin, B., Costalat, G. and Renshaw, G. M. C. (2022) Pre-acclimation to altitude in young adults: choosing a hypoxic pattern at sea level which provokes significant haematological adaptations. *European Journal of Applied Physiology* **122**, 395-407. https://doi.org/10.1007/s00421-021-04837-8
Tsukiyama, Y., Ito, T., Nagaoka, K., Eguchi, E. and Ogino, K. (2017) Effects of exercise training on nitric oxide, blood pressure and antioxidant enzymes. *Journal of Clinical Biochemistry and Nutrition* **60**, 180-186. https://doi.org/10.3164/jcbn.16-108
Turner, G., Gibson, O. R., Watt, P. W., Pringle, J. S. M., Richardson, A. J. and Maxwell, N. S. (2017) The time course of endogenous erythropoietin, IL-6, and TNFα in response to acute hypoxic exposures. *Scandinavian Journal of Medicine and Science in Sports* **27**, 714-723. https://doi.org/10.1111/sms.12700
US Department of Health and Human Services (2019) *US Department of Agriculture 2015-2020 Dietary Guidelines for Americans*. Washington, DC, USA: US Department of Health and Human Services.
Vinet, A., Mandigout, S., Nottin, S., Nguyen, L., Lecoq, A.-M., Courteix, D. et al. (2003) Influence of body composition, hemoglobin concentration, and cardiac size and function on gender differences in maximal oxygen uptake in prepubertal children. *Chest* **124**, 1494-1499. https://doi.org/10.1378/chest.124.4.1494
Vogt, M., Puntschart, A., Geiser, J., Zuleger, C., Billeter, R. and Hoppeler, H. (2001) Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *Journal of Applied Physiology* **91**, 173-182. https://doi.org/10.1152/jappl.2001.91.1.173
Wilber, R. L. (2007) Application of altitude/hypoxic training by elite athletes. *Medicine and Science in Sports and Exercise* **39**, 1610-1624. https://doi.org/10.1249/mss.0b013e3180de49e6
Wojan, F., Stray-Gundersen, S., Nagel, M. J. and Lalande, S. (2021) Short exposure to intermittent hypoxia increases erythropoietin levels in healthy individuals. *Journal of Applied Physiology* **130(6)**, 1955-1960. https://doi.org/10.1152/japplphysiol.00941.2020
World Health Organization (2018) Global database of body mass index: BMI classification. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html
Yamashita, K., Discher, D. J., Hu, J., Bishopric, N. H. and Webster, K. A. (2001) Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, and p300/CBP. *The Journal of Biological Chemistry* **276(16)**, 12645-12653. https://doi.org/10.1074/jbc.M011344200
Zhang, Q., Zhao, W., Li, S., Ding, Y., Wang, Y. and Ji, X. (2023) Intermittent hypoxia conditioning: A potential multi-organ protective therapeutic strategy. *International Journal of Medical Sciences* **20(12)**, 1551-1561. https://doi.org/10.7150/ijms.86622
Żebrowska, A., Jastrzębski, D., Sadowska-Krępa, E., Sikora, M. and Di Giulio, C. (2019) Comparison of the effectiveness of high-intensity interval training in hypoxia and normoxia in healthy male volunteers: A pilot study. *BioMed Research International* **2019**, 7315714. https://doi.org/10.1155/2019/7315714

Key points

- High intensive interval training in hypoxia significantly enhances aerobic performance in young athletes, providing valuable evidence for optimizing training regiments.
- Training in hypoxia has the potential to increase protective mechanisms against exercise-induced motor performance fatigue compared to the normoxia training.
- Exposure to hypoxia modulates the cytokine and inflammatory processes with a non-significant effect on antioxidant status.

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